NL Neurology Letters

Review Article

https://doi.org/10.52547/nl.2.1.16

Mesenchymal stem/stromal cell-derived extracellular vesicles in treatment of neurodegenerative diseases

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Abstract

Mesenchymal stem cells (MSCs) are multipotent mature stem cells found in a variety of organs. Their unique therapeutic efficacy has made them an attractive choice in regenerative therapy thus far. MSCs and MSC-derived substances have been used to treat neurodegenerative diseases (NDs) such as Alzheimer's disease (AD), Huntington's disease (HD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and multiple sclerosis (MS). Over the last few decades, tremendous progress has been made in understanding the potential therapeutic remedies and etiology of numerous NDs. One of the MSC-based therapies is the application of extracellular vesicles (EVs), which involves a variety of physiologically active components such as proteins and nucleic acids. The contents of EVs are transported across cells for intercellular interaction, that usually leads to recipient cell homeostasis regulation. EVs are a feasible cell-free therapy option for tissue regeneration and repair since they are the therapeutically advantageous cargo of parent cells and are free of many ethical issues associated with cell-based therapies. However, the paucity of standard methodologies in EV extraction processes, as well as their pharmacological properties and mechanism of action, have made developing novel EV-dependent therapies challenging. This article reviews studies on MSCs cell-derived EVs in the treatment of NDs.

Keywords: Mesenchymal stem/stromal cell-derived extracellular vesicles, neurodegenerative diseases treatment, MSCs-based therapy

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Published online 30 April 2023

Cite this article as:

1. Introduction

Mature stem cells called mesenchymal stem cells (MSCs) can be found in practically every post-natal organ and tissue, like the brain. MSCs naturally produce a variety of active ingredients with trophic, immunomodulatory, anti-inflammatory, anti-apoptotic, pro-angiogenic, and pro-regenerative properties. MSCs regulate immune function in a paracrine manner by producing immunomodulatory factors such as hepatic growth factor (HGF), transforming growth factor (TGF), interleukin (IL)6, and, IL-10, as well as pro-angiogenic factors like basic fibroblast growth factor (bFGF) and placental growth factor (PGF) (1). Extracellular vesicles (EVs), which are released by cells, have recently been revealed to play an important role in cell-to-cell communication. Exosomes, microvesicles (MVs), and apoptotic bodies (APBs) are all examples of EVs (2). MSC-derived EVs (MSC-EVs) have been found to provide therapeutic benefits comparable to their whole-MSC counterparts, demonstrating that EVs are essential mediators of MSC treatment efficacy. These nano-sized vesicles have sparked optimism in curing intractable neurodegenerative illnesses owing to their ability to carry a broad range of biomolecules across a long distance among cells and to overcome biological barriers (3-7). Behavioral or cognitive dysfunction, as well as deficits in remembering, thinking, and reasoning, are all symptoms of neurocognitive illnesses, which arise as a result of gradual neuronal loss and degeneration (8). Neurodegenerative diseases (NDs) are a set of neurological disorders marked by nervous system malfunction and gradual neuronal loss that are linked to aging. Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS),

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Huntington's disease (HD) are the most frequent NDs. These disorders progress over time and are usually accompanied by the buildup of protein aggregation, the composition of which varies depending on the condition (9). Here, we discuss some current aspects regarding the therapeutic potential of MSC-EVs in the treatment of AD, HD, PD, ALS, and MS.

EVs types and biogenesis

EVs are a collection of nano-sized vesicles with varying sizes, contents, and surface markers that are released into the extracellular environment by a number of methods and transport cytoplasm and cell membrane components that are specifically placed into these vesicles. They are produced by every living cell and play important roles in a variety of physiological and pathological processes (10). Exosomes, MVs, and ABs are all examples of EVs, according to the International Society of Extracellular Vesicles (ISEV) (2). This classification is based on their diameter size and origin; hence, each subclass of EVs reflects distinct physicochemical features that play critical roles in both normal and pathological circumstances (11). MVs, also known as ectosomes, are the first type of vesicle formed by direct budding of vesicles from the cell membrane to the cell's exterior (12). Exosomes are the second kind, which develop by budding into endosomes to form what are known as multivesicular bodies (MVBs). These MVBs either bind to lysosomes, causing the content to be digested, or they bind to the cell membrane, causing the material to be discharged as exosomes (13). Exosomes are 50 to 150 nanometers in diameter, while MVs are 100 to 1000 nanometers in diameter (14). Apoptotic bodies (APBs), the third kind, are formed when cells undergo apoptosis. APBs are formed by the separation of membrane blebs or by the formation of apoptopodia during the apoptotic process. Beaded apoptopodia and Non-beaded apoptopodia are the two forms of apoptopodia(15). APBs have a significantly greater range of diameters, ranging from 50 to 5000 nm (16).

1.1 Biogenesis of exosomes

Exosomes are an endosomal-derived subtype of EV. Exosomal vesicles are formed by the inner budding of early endosomes' limiting membranes, which grow into MVBs throughout the process (14, 17). They are formed when the plasma membrane is doubly invaginated and intracellular MVBs containing intraluminal vesicles (ILVs) are formed (18). ILVs are eventually released as exosomes as a result of MVB fusion to the plasma membrane and exocytosis. The initial invagination of the cell membrane results in the formation of a cup-shaped structure containing both cell-surface proteins and soluble proteins from the external environment. This results in the development of an early-sorting endosome (ESE) from scratch, which in some situations may combine with an existing ESE (19). The ESE's development and composition can also be influenced by the endoplasmic reticulum and trans-Golgi network (13, 20, 21). MVBs, also known as multivesicular endosomes, form as ESEs develop into late-sorting endosomes (LSEs). The endosomal limiting membrane is invaginated inward, forming MVBs, which are double invaginations of the cell membrane. MVBs with several ILVs are created as a result of this procedure (future exosomes). The MVBs can either merge with lysosomes or auto-phagosomes and be destroyed, or it can fuse with the cell membrane and release the ILVs housed therein as exosomes (19, 22). The Sytenin-1, Ras-related protein GTPase Rab, tumor susceptibility gene 101(TSG101), syndecan-1, apoptosis-linked gene 2-interacting protein X (ALIX), endosomal sorting complexes required for transport (ESCRT), tetraspanins, phospholipids, sphingomyelinases, ceramides, and soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor (SNARE) are involved in the biogenesis and origin of exosomes, but their precise rate-limiting actions and functions in exosome biogenesis require further investigation, particularly in vivo(20, 23, 24).

1.2 Biogenesis of apoptotic bodies

Dying cells produce apoptosis bodies throughout the extracellular environment. When apoptotic cells are broken down into ApoBDs, one of the first and most noticeable morphological alterations is cell deformation, which manifests as membrane blebbing. Blebs occur as the result of increased hydrostatic pressure within the cell in response to actomyosin-mediated contraction (25). The repetitive blebbing culminates in the formation of ApoBDs, which are filled with organelles and other cell components like as chromatin (26). Another process involved in the production of apoptotic bodies is apoptotic volume decrease (AVD). It is a preliminary event that takes place in conjunction with membrane blebbing, culminating in the shrinkage of apoptotic cells (27, 28). Membrane deformation occurs in a variety of ways in various cell types, including membrane bulges like apoptopodia, beaded apoptopodia, and microtubule spikes. ApoBD creation is accelerated by beaded apoptopodia, which is a novel mechanism (15).

1.3 Biogenesis of microvesicles

EV can pinch off and bud the plasma membrane in addition to MVB-derived exosomes. They are commonly referred to as MVs, despite the fact that their sizes range from exosome-like EVs of 50 nm to EVs as large as 10 μm. MVs can be produced in a variety of ways, some of which partially overlap with those participating in exosome formation, owing to their heterogeneity. The ESCRT machinery, for example, is engaged in the generation of nanometer-sized vesicles that are abundant in cell surface proteins, indicating that they originate from the cell membrane (29, 30). Moreover, acid sphingomyelinase causes ceramide-dependent microvesicle production, comparable to the function of nSMase2 in ILV creation, implying a universal role for sphingomyelinases in the synthesis of MVB- and cells membrane-derived EVs (31). Non-apoptotic cellular membranes blebs, which are common in highly aggressive cancer cells, are connected to another method of microvesicle formation. These blebs constantly expand and contract at the cell surface, facilitating cell mobility by an unknown process (32). Furthermore, actin cytoskeleton conformational changes at the vesicle budding

tip can lead to membrane scission, allowing these blebs to be discharged as MVs (33-36). Tumor cells can assume an amoeboid morphology during migration in vivo, which is linked with significant cell membrane blebbing and microvesicle release, implying a function for this form of EV biogenesis during cancer metastasis and invasion (23, 36-38).

2. EVs characterizing

Cells produce lipid-bound vesicles into the extracellular environment, which are referred to as EVs (17, 39). MicroRNAs (miRNAs), mRNAs, circular RNAs (circRNA), long noncoding RNAs (lncRNA), proteins, lipids, and metabolites are all possible components of EVs (40, 41). APBs also include entire cell organelles such as mitochondria, endoplasmic reticulum, and ribosomes (16).

2.1 Exosome

Dr. Rose Johnstone invented the word exosome. Exosomes, also known as ILVs, are released by all types of cells and have been detected in urine, plasma, sperm, bronchial fluid, saliva, cerebral spinal fluid (CSF), serum, breast milk, amniotic fluid, tears, synovial fluid, lymph, stomach acid, and bile (42-46). Exosomes include a diverse range of lipid-anchored membrane proteins, transmembrane proteins, exosome lumen soluble proteins, and peripherally associated membrane proteins. Exosomes have a role in cell survival, cell-cell communication, and tumor growth. They have been shown to promote immunological responses by serving as antigen-presenting vesicles (47, 48). Exosomes have been reported to aid in neurite growth, production of myelin, and neuronal survival in the nervous system, hence aiding in regeneration and tissue repair (49-53).

2.2 Microvesicles

MVs (also known as microparticles or ectosomes) are EVs that are discharged from cell membranes (39). Megakaryocytes, the placenta, monocytes, blood platelets, tumor cells, and neutrophils, are all sources of MVs (54). They can be detected in both tissues and bodily fluids (55). The parental cell's cellular membranes phospholipid asymmetry has been eliminated in microvesicles, and a phospholipid bilayer acts as a barrier (56-58). MVs help cells communicate with one another by transporting chemicals including proteins, miRNA, and mRNA between cells (59). MVs, which were once discarded as cellular trash, may now reflect the antigenic composition of the cell of origin and play a role in cell signaling. Tumor immune suppression, anti-tumor effects, metastasis, angiogenesis, tissue regeneration, and tumor-stroma interactions have all been linked to EVs (60-63). MVs can also help cells get rid of misfolded proteins, cytotoxic chemicals, and metabolic waste. Variations in MVs level can signal a variety of illnesses, including cancer (64, 65).

2.3 Apoptotic bodies

APBs are created exclusively when programmed cell death takes place (66, 67). Smaller vesicles are indeed produced during this activity, which are distinguished by the existence of organelles inside the vesicles (68). MVs and exosomes have a completely different makeup than APBs. Unlike MVs and exosomes, ABs include intact organelles, a minor quantity of glycosylated proteins, and chromatin (14, 69). As a result, greater amounts of proteins linked with the mitochondria (HSP60), nucleus (histones), golgi apparatus, and endoplasmic reticulum (i.e., GRP78) are anticipated. Furthermore, the proteome profiles of cell lysate and APBs are quite similar, but the proteomic patterns of cell lysate and exosomes are very different (70, 71). Phosphatidylserine (PS) is indeed the sole ApoBD marker that has been found thus far (15).

3. MSCs-EVs component

3.1 Pro-angiogenic factors

Angiogenesis refers to the formation of new capillaries from previously existing blood vessels (72, 73). MSC-EVs are both anti- and pro-angiogenic (74). Vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), tumor necrosis factor-alpha (TNF-α), TGF-β, and angiopoietins are some of the most well-known angiogenic growth factors and cytokines. Endothelial cells, fibroblasts, smooth muscle cells, platelets, inflammatory cells, and cancer cells are all the main sources of these growth factors (75).

3.2 Immunomodulatory factors

MSC-EVs have Immunomodulatory activity and contain chemicals that affect immune cells. They include inflammatory cytokines and chemokines, that can influence both innate (e.g., macrophages, dendritic cells, and natural killer (NK) cells) and adaptive immune cells (T and B cells) (76, 77) (Fig 1). Overexpression and knockdown procedures were employed by several research groups to discover the active immunomodulatory molecules essential for MSC-EVs' treatment efficacy (78-86). Eirin et al. discovered that MSC-EVs carrying IL-10 improved renal shape and function, reduced renal inflammation, and increased the number of reparative macrophages in pigs with renal artery stenosis (RAS). When EVs derived from MSCs with knocked-down IL-10 were employed to treat RAS, these advantages were completely lost (78).

In bronchopulmonary dysplasia mice, MSC-EVs releasing TSG-6 protein, an immunomodulatory protein encoded in pathological settings in response to rising inflammation, were effective in reducing lung inflammatory process and cell death. However, the therapeutic efficacy was lost when MSC-EVs without TSG-6 expression were utilized (87). Some other studies found that MSC-EVs harboring the C-C motif chemokine receptor-2 (CCR2) might prevent monocyte and macrophage activity and protect mice from renal/ischemia harm. Several studies have also shown that particular miRNAs that are overexpressed in MSC-EVs play an immunomodulatory role (88). In this regard, MSC-EVs containing miRNAs such as miR-142-3p, miR-21-5p, miR-126-3p, and miR-223-3p are endowed with the capacity to regulate dendritic cell maturation and boost their anti-

inflammatory activity. Likewise, in a polymicrobial sepsis mouse model, MSC-EVs carrying miR-223-3p were able to diminish pro-inflammatory cytokine release in macrophages, systemic inflammation response, heart failure, and increase survival. These positive therapeutic effects were not observed in MSC-EVs of the miR-223-3p mutant mouse. MSC-EVs expressing miR-223, on the other hand, have been demonstrated to protect against liver impairment in autoimmune hepatitis types and to down-regulate a number of cytokines and inflammatory genes (89-91). MSC-EVs harboring miRNAs including miR-146a, miR-21a-5p, miR-223, and miR-199a regulated several inflammatory genes (e.g., NLRP3 and IL-6), and promoted macrophage polarity toward the anti-inflammatory M2 phenotype (92).

3.3 Anti-apoptotic factors

MSCs have anti-apoptotic properties. According to an experimental investigation, the conditioning medium of humans MSC culture has a paracrine anti-apoptotic impact on hypoxia-induced death of rat lungs alveolar cells. One of MSCs' anti-apoptotic capabilities is the production of HGF and keratinocyte growth factor, which inhibit pro-apoptotic signals induced by reactive oxygen species (ROS) and hypoxia-inducible factor-1 alpha (HIF-1) (1). MSCs simply release biologically active proteins and EVs that activate endogenous lung stem/progenitor cells, causing them to proliferate and differentiate, impede apoptosis, reduce inflammation, reestablish capillary barrier activity, and decrease fibrosis. They can treat both acute and chronic lung injuries since they function similarly to parental MSCs (93, 94). As a result, MSC-derived secretomes have anti-apoptotic properties, resulting in improved tissue healing and regeneration (95, 96). Several recent investigations have found that the secretome of MSCs can regulate apoptosis in both healthy and pathological settings. MSC-derived conditioned medium (MSC-CM) therapy reduced pro-apoptotic cleaved caspase-3 and Bax expression in parenchymal cells while increasing anti-apoptotic B-cell lymphoma/leukemia-2 (Bcl-2) synthesis, avoiding their elimination during chronic inflammation. Researchers showed that injection of MSC-CM promoted liver regeneration and raised survival rate in research mice by lowering inflammatory cell infiltration in the inflammatory liver and dampening apoptosis and promoting the proliferation of wounded hepatocytes (97-101). MSC-CM induced inhibition of apoptosis and improved regeneration of damaged hepatocytes was predominantly mediated via HGF, fibrinogen-like protein 1, IDO-1/KYN, and IL-6/gp130 signaling pathways, among a variety of MSC-derived trophic, and immunomodulatory hepatoprotective components. MSC-Exos reduced a negative immunological response and death of hepatocellular in ALF and blocked TGF- synthesis in HSCs, leading to a reduction of liver fibrosis in a way similar to MSC-CM (95, 102-105).

4. MSCs-EVs in Alzheimer's disease

AD is a kind of dementia that typically affects the elderly. This neurodegenerative condition gradually and irreversibly impairs brain functioning (remembering, reasoning, and thinking), thought content, personality, and behavior (106). The most widely acknowledged scientific explanations for pathological aspects of AD include extracellular aggregation of beta-amyloid peptide (Aβ), formation of neurofibrillary tangles (NFT) due to intracellular depositing of hyperphosphorylated tau, and persistent neuroinflammation (107). Neurotic communication abnormalities and the loss of individual neurons are caused by aberrant protein accumulations outside and inside nerve cells (108). The deposition of Aβ, the major component of amyloid plaque, within neurons is thought to be responsible for the initial stages of AD pathology (109). These findings highlight the significance of exosomes in the progression of AD via the distribution of Adeposits(110). Several studies have evaluated the potential of exosomes like a biomarker for early diagnosis of AD or as a delivery vehicle for therapeutic compounds including tiny medicines, miRNA, and siRNA (111, 112). For instance, Saman et al. employed tau-containing exosomes generated from CSF for the initial diagnosis of AD (113). Furthermore, since CSF-derived exosomes contain both p-tau and Aβ, the discovery of both possible biomarkers in exosomes may indeed raise the value of the currently employed marker for the initial detection of AD (114, 115). In this regard, Clark et al. discovered that using a combination of markers to diagnose AD enhanced specificity and sensitivity by 86% (116-119). According to a study, AD sufferers' CSF-derived exosomes had greater levels of miR-598 and miR-9-5p, than their healthy counterparts (120). Plasma, on the other hand, has long been evaluated to distinguish markers; hence, plasma seems to be a more convenient and accessible option (121). Furthermore, the biological components of exosomes have demonstrated great accuracy for the initial detection of AD (122). When comparing plasma exosomal protein expression among AD cases and healthy controls, researchers discovered that patients with AD had higher levels of neuron-derived proteins such as Aβ and overall tau (115).

Several investigations have confirmed the neuroprotective properties of neuron-derived exosomes. Exosomes generated from glia, for instance, protect neurons from oxidative stress (53). The findings of a study into the processes associated with exosomes in Aβ clearance showed that neuron-derived exosomes injected into the brains of AD transgenic mice assisted in Aβ peptide elimination via two key mechanisms. Because of conformational alterations, the exosomal surfaces prion receptor can bind to amyloid plaques and transform them into harmless forms. Exosomes also speed up microglia absorption of Aβ extracellular plaque. Furthermore, many of the investigations focused on using exosomes released by MSC for medicinal purposes (123, 124). Exosomes released by MSC derived directly from connective tissue, such as bone marrow and adipose tissue, not just successfully cross the blood-brain barrier (BBB), but also successfully cleave intracellular and extracellular Aβ peptide in the brain attributed to the prevalence of neprilysin enzymes (125, 126). MSC-EV interacts with Aβ directly through its lipid membranes, increasing plaque phagocytosis via microglia. MSC-EVs also transport neprilysin, a protease that breaks down Aβ, lowering

Table 1. Types of vesicles

Vesicles	Size	Origin
Apoptotic bodies	(50—500 nm) in diameter	Outward blebbing of apaoptotic cell membrane
Exosoms	(30 —200 nm) in diameter	Luminal budding into MVBs;release by fusion of MVB with cell membrane
Microvesicles	50—1000 nm	Outward budding of cell membrane

intracellular Aβ deposition indirectly. So far, in vivo studies examining MSC-EV therapeutic effects in AD models have relied on long-term therapy (weeks/months), with EVs being administered through systemic pathway (127) or intracerebroventricular (ICV) (128, 129), demonstrating either partial recovery (127, 128) or a protective function in diminishing the burden of Aβ plaque and the number of dystrophic neurites (129).

Further studies found that injecting MSCs or MSC-derived EV into hippocampal neurons boosts their resistance to the Aβ-induced synaptic degradation and deleterious impacts of oxidative stress (130). De Godoy and colleagues suggested multiple mechanisms, including reduced extracellular Aβ due to MSCs' elevated endocytic potential, secretion of EVs usually contains antioxidant enzymes like catalase, and paracrine activity via extracellular eventual release of trophic factors and anti-inflammatory cytokines like VEGF, IL-6, and IL-10. Despite the fact that MSC-EVs have been utilized to treat AD in the majority of studies to date, two latest studies suggest that different stem cell sources have medicinal ability to develop AD-induced mental disorder through different processes such as lowering extracellular and intracellular Aβ deposition oligomer (130). Recently, Sha et al. reported new avenues for understanding the therapeutic mechanism of MSCs in AD. They demonstrated that bone marrow MSC-EVs carried miR-29c-3p to neurons, inhibiting expression of beta-secretase (BACE1) and stimulating the Wnt/-catenin pathway, culminating in a therapeutic effect in AD (131).

Wang et al. evaluated the effect of MSC-EVs on neuronal impairments in Aβ-stimulated hippocampal neurons, or AD cell (SHSY5Y cell lines), as well as animal models (APPswe / PS1dE9 mice) (132). Their findings revealed that treatment with MSC-EVs alleviated cognitive deficits and decreased aggregation of hippocampal Aβ and neuronal loss in mice with AD. They also discovered that the nuclear factor-erythroid factor 2-related factor 2 (Nrf2) signaling pathway was involved in the effects of MSC-EVs in cell lines and mice models. These findings point to MS-EVs as prospective nanotherapeutics for restoring hippocampal neuronal form and activity in APP / PS1 mice, underlining the clinical utility of MSC-EVs in AD therapy. According to the above concepts, MSC-derived EVs have beneficial effects on AD(132). Diverse stem cell sources show therapeutic potential to alleviate AD-induced cognitive deficits through multiple pathways such as lowering extracellular and intracellular Aβ deposition oligomers, despite the fact that MSC-EV has been employed to cure AD in the majority of research to date.

5. MSCs-EVs in Parkinson's diseases

PD is the world's second most popular persistent neurodegenerative illness, having been first identified by James Parkinson around 1817(133). Although the underlying mechanisms of PD remain unclear, both hereditary and environmental variables have a role in the PD pathogenesis (134, 135). The deterioration of dopaminergic neurons (DAn) and a lack of dopamine synthesis in numerous dopaminergic networks are pathological features of PD. The accumulation/formation of Lewy bodies (protein clumps of -synuclein) in the neurotic structure is associated with the elimination of dopaminergic neurons, impairing the regular functioning of those cells. The nigrostriatal route at the levels of the substantia nigra pars compacta (SNpc) and the striatum (STR) (133) is the main impacted network, with such an asymmetrical start that's become bilateral even as illness develops (136). Exosomes can deliver catalytically functional enzymes like phosphatase and tensin homolog (PTEN) and biologically active lipids including prostaglandins to target cells (137, 138). Exosomes include a variety of proteins known as "exosome markers", the majority of which are involved in their production. They also transport transmembrane molecules that can aid inside this immunoselection of such exosomes with a specific biological origin, boosting exosome sensitivity as biomarker. Exosomes have been found to contain misfolded proteins in neurodegenerative illnesses, such as α-synuclein (α-syn) in PD (58, 139). The presence of genetic material, such as miRNAs, is one of the largest common contents of exosomes (74).

Several disorders, including PD, have been shown to have severe deregulation of gene expression, particularly at the miRNA level (140). Multiple studies have shown that MSC-derived exosomes may transfer miRNAs to neuronal cells, with miR-133b-rich exosomes promoting neurite outgrowth, which is beneficial for PD because this is one of the miRNAs that is generally downregulated in the PD (141). Despite this, miR-143 and miR-21 were detected in MSC-derived exosomes, and have been described as significant participants in immune response regulation and neuronal loss linked with a chronic inflammatory milieu (142). Numerous experiments revealed that α-syn is either released directly into the extracellular space or even packaged into exosomes (143). Furthermore, α-syn has been shown to interface with synaptic

Table 2. The use of extracellular vesicles produced from stem cells in neurodegenerative disorders.

Disease	Type of EVs and origin	Outcomes	Ref
AD	MSCs/exosomes	Encourage neurogenesis and the restoration of cognitive function.	[193]
PD	MSCs/exosomes	transferring of the miR-133b regulates neurite outgrowth.	[141] [194]
	MSCs/exosomes	Improved neuronal function and oligodendrogenesis stimulation	
MS	MSCs/EVs	Drive peripheral resistance, activate apoptotic signaling pathway in self-reactive lymphocytes, and stimulate regulatory T cell differentiation by:	[164]
		- IL-10 and TGF-β secretion	[167]
		-expression of PD-L1 and TGF-β	
	MSCs/exosomes	Reduce CNS inflammation and demyelination by doing the following: - shifting microglial polarization toward an M2 phenotype	

vesicles to enhance neurotransmitter release and SNARE assembly. The Golgi or clathrin-mediated endocytosis can arrange synaptic vesicles usually containing α-syn into early endosomes (144, 145). Then, α-syn containing endosomes convert into MVBs and merge with the cell membrane before being released as exosomal payloads, thanks to the help of VPS4 and small ubiquitin-like modifier (SUMO) proteins. In a Rab11a-dependent manner, α-syn containing endosomes also can be straightened into recycling endosomes and exocytose as secretory granules (146, 147). The cytosolic calcium concentration regulates the release of exosomal α-syn from living cells in all procedures. Despite the fact that the rates of α-syn in exosomes are minimal, recent research suggests that exosomes include an ideal environment for α-syn to accumulate and possibly promote the spread of PD pathology. It is widely assumed that oligomeric α-syn is indeed the harmful substance of α-syn that causes neuronal cell death (148, 149). Nowadays, PD is usually detected by the manifestation of visible clinical motor symptoms. There is no useful diagnosis for early-stage PD at the moment. The advancement of a method for early detection of PD would be a significant step forward. Previous research has found that disorder components of EVs (including exosomes) isolated from the blood or CSF of PD patients can be effective biomarkers of the disease (150-153). Understanding the intricacy of exosomes produced from MSCs, as well as how their miRNA contents interact with cellular and molecular PD pathways, is crucial.

6. MSCs-EVs in Multiple sclerosis

MS is an inflammatory demyelination of the central nervous system (154). In addition to inflammation and demyelination in the spinal cord and brain, other pathological biomarkers of MS include BBB disruption, reactive gliosis, oligodendrocyte loss, and neuron and axonal degeneration (155). According to the National MS Society, the four major types of MS are primary progressive MS (PPMS), secondary progressive MS (SPMS), relapsing-remitting MS (RRMS), and clinically isolated syndrome (CIS) (NMSS)(156). According to Liu and his colleagues, the primary explanation for stem cell immunomodulatory activities is HLA-G expression, which operates as an inhibitor of NK death in response to interferon-gamma (IFN-γ), the principal inflammatory mediator implicated in the development of MS (157). This strategy was capable of achieving long-term, if not permanent, remission in MS patients who were not receiving treatment for their disease. Even though autoimmune attacks are the most significant reason for axon demyelination in illnesses like MS. Ideally, tissue restoration via stem cell transplantation may lead not only to axon reconstruction by replacing lost and destroyed cells, but also to anti-inflammatory and paracrine neuroprotective effects, potentially preventing progressive neural and axonal degeneration (158, 159).

Rajan et al. found that conditioned medium containing exosomes generated from periodontal ligament stem cells had anti-inflammatory and suppressive properties in experimental autoimmune encephalomyelitis (EAE) mice models of MS. These findings revealed that an increase in anti-inflammatory cytokines, such as IL-10, leads to considerable remyelination in the spinal cord and reversing MS progression. Impaired T cell activation, which plays a major role in the control of T

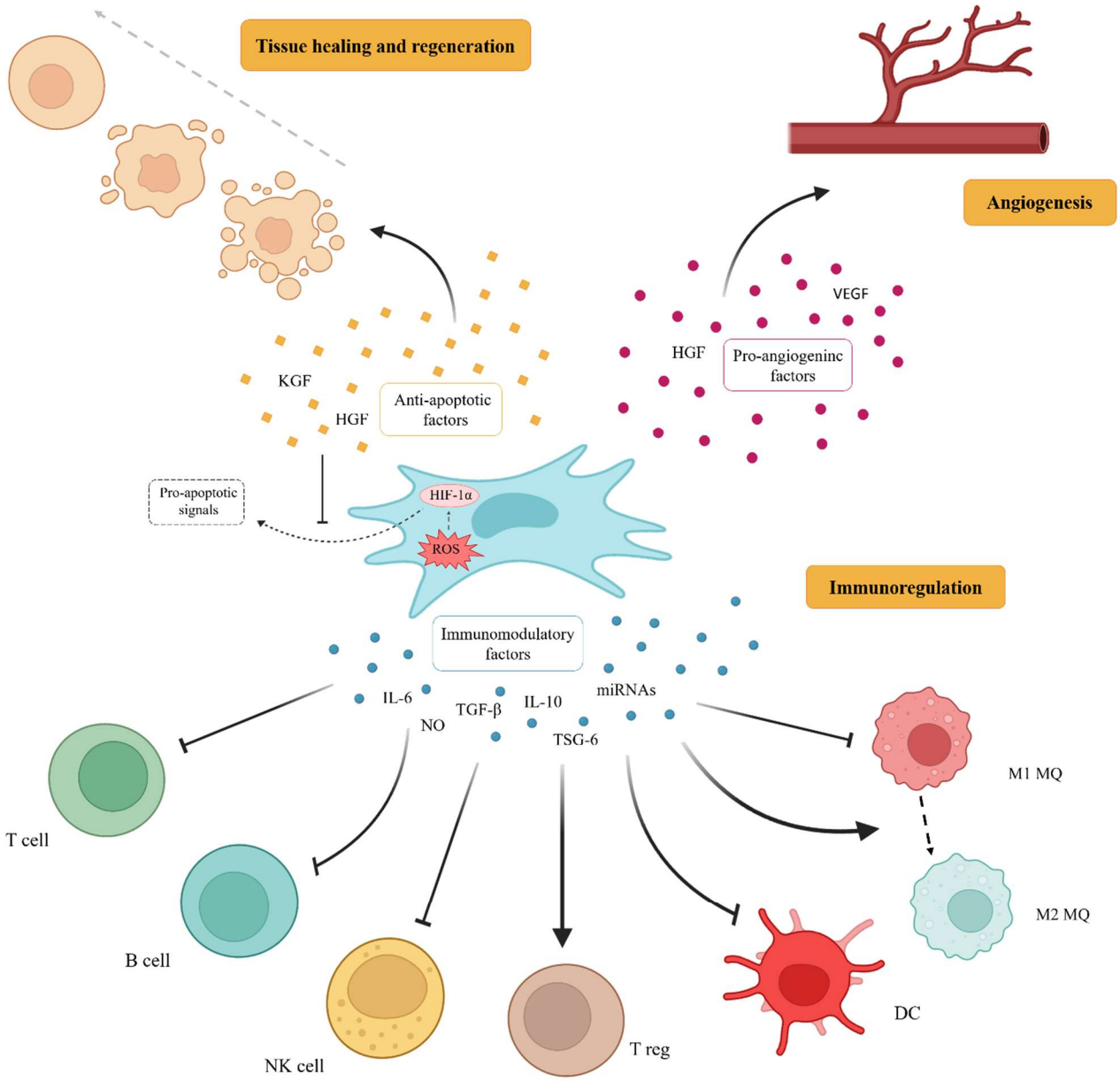

Figure 1. Different physiologic functions of MSCs through secretory factors (secretoms) or through cell-to-cell contact.

MScs simply release EVs that can impede apoptosis (anti-apoptotic features), reduce inflammation (immune-regulation features), and reestablish capillary barrier activity (pro-angiogenic features). Altogether, EVs have a beneficial function in the treatment of acute and chronic injuries because they operate similarly to parental MSCs.

Abbreviation: HGF: hepatocyte growth factor, KGF: keratinocyte growth factor, HIF-1a: Hypoxia Inducible Factor 1 Subunit Alpha, ROS: Reactive oxygen species, MSC: mesenchymal stem cell, TGF: transforming growth factor, TSG: tumor susceptibility gene, miRNA: microRNA, VEGF: vascular endothelial growth factor

helper (Th)1/Th2 balance, is one of the pathogenic characteristics of MS (160). Clark et al. Found that EVs produced by placental-derived mesenchymal stem cells (PMSCs) could achieve therapeutic effects similar to individual EAE therapy if administered in high doses. Also, according to VEGF proteomic analyses, the HGF was found in EVs produced from PMSC. PMSCs regulate the immune system by triggering regulatory T cells (Tregs) with large amounts of these substances that they release. This discovery indicated that PMSC-EV can stimulate myelin regeneration and elicit immunomodulatory effects comparable to PMSC therapy in the EAE mice model (161). Similarly, intravenous treatment of MSC-EVs derived from human adipose tissue enhances the score of EAE animals by preventing immune cell infiltration, regulating their activation, and lowering the production of pro-inflammatory cytokines (162).

Induction and maintenance of immunological tolerance is a major goal in the treatment of autoimmune conditions. Maintenance and progression of regulatory molecules like TGF-β, programmed death ligand-1 (PD-L1), and galectin-1 via biological interventions in the host immune system is one of the most current approaches for peripheral tolerance (162, 163). Mokarizadeh et al. investigated Tolerance of MV contents produced by MSC to increase environmental resistance in splenic mononuclear cells (MNCs) based on EAE mice. MSCs-MV activates apoptotic signaling in self-reactive lymphocytes, stimulating them to secrete IL-10 and TGF-β, as well as increasing the expression of regulatory molecules such as TGF-β on and PD-L1 on the MV surface, leading to the differentiation of Tregs that prompt peripheral tolerance of immune responses (164).

Another emerging method for establishing immunological tolerance in MS patients is the polarization of microglia toward the M2 phenotype (165). Microglia are the CNS's resident macrophages that are rapidly activated by microenvironments (like infection, ischemic injury, and pro-inflammatory cytokines such as TNF-α, IL-1β) to make a differentiation either into the M1 phenotype, which causes CNS damage and generates pro-inflammatory cytokines, or the M2 phenotype, which fosters tissue regeneration by producing anti-inflammatory cytokines(166). In the early stages of MS, an imbalance of M1/M2 macrophages and a shift toward pro-inflammatory M1 phenotypes was considered to be one of the major drivers to tissue injury in the CNS. As a result, it is thought that prompting microglia to polarize toward the M2 phenotypes might improve MS patients' neurological symptoms (165). In this regard, Li et al. investigated the impact of BMSC paracrine pathways, namely exosome mediation, on microglial polarization and motor functional improvements in an EAE mouse model (167). They found that exosomes derived from BMSC can decrease demyelination and inflammation of the CNS while improving neural behavioral ratings in the EAE animal model via shifting the polarity of microglia toward an M2 phenotype. Furthermore, MSC exosome therapy decreased M1-associated TNF and IL-12 levels while increasing M2-associated cytokines (IL-10 and TGF-β) (167, 168). Exosomes can carry medications to MS patients because of their capacity to cross the BBB. Diverse functional elements to the surfaces of exosomes, such as aptamers and antibodies, dramatically improves the exosomes' specificity (119). Based on these findings, it's safe to say that MSC-EVs will represent the future the MS therapy approaches for various reasons, such as their safety, capacity to cross the BBB.

7. MSCs EVs in Amyotrophic lateral sclerosis

ALS is a deadly adult-onset neurodegenerative disease that was discovered in the 1870s. There is a family background of ALS in 5%–10% of individuals (familial ALS, fALS), however there is no apparent genetic relationship in 90%–95% of patients (sporadic ALS, sALS) (169, 170). After 2 to 5 years after the onset of the disease, the patient's condition becomes severe and fatal. Both sALS and fALS are characterized by the selective loss of higher motoneurons in the primary motor cortex, as well as lower motoneurons in the brainstem and spinal cord. Some motoneurons, such as those that control pelvic muscles (Onuf's nucleus in the sacral spinal cord) and eye movements, are unaffected by the condition (oculomotneurons). It's unclear what causes this difference in motoneuron susceptibility (171).

Neurodegeneration appears to have a complex pathophysiological mechanism with multiple pathways involved. The increasing influx of Ca2+ ions inside the motoneurons is caused by a rise in the neurotransmitter glutamate in the synaptic cleft (glutamate excitotoxicity) induced by astrocyte uptake dysfunction. Because of mitochondrial dysfunction, elevated Ca2+ ions remain high in the cytoplasm and can develop dementia by activating Ca2+-dependent enzyme pathways that contribute to oxidative stress. Mutation misfolding proteins formation intercellular aggregates, enhance oxidative stress, contribute to mitochondrial impairment, and may lead to an excess of neurofilaments (NFs) and axonal transport dysfunction. Furthermore, neurotoxicity is exacerbated by the release of inflammatory mediators and toxic substances by activated astrocytes and microglia (172). Exosomes and MVs have received increased attention as boosters and suppressors of disease processes due to their ability to transmit biological information across large distances. Exosomes derived from primary neurons or neuroblastoma have been shown to improve the course of AD in a mouse model by sequestering intracerebral (173). After oxidative stress, the neuroprotective effect of exosomes produced from adipose-derived stromal cells (ASC) was also established in primary murine hippocampus neurons and human neuroblastoma cells. Exosomes derived from BM-MSC have also been used to aid recovery and neuroregeneration following strokes and traumatic brain injuries. Exosomes derived from ASC have recently been shown to protect neurons in an in vitro model of ALS (7, 174, 175). The motoneuron-like cell line NSC-34 was stably and transiently transfected with various SOD1 point mutations to imitate the behavior of ALS motoneurons, and H2O2 was employed as a pathogenic insult in the investigation. Exosomes boosted ALS motoneuron lifespan by blocking the apoptotic pathway, implying that they could be used as a treatment for the disease. Another study showed that ASC-derived exosomes could be used to treat ALS by lowering mutant SOD1 improving and aggregating mitochondrial protein activity (176). Although the researchers still have a long path to go, the recent discovery on EVs gives patients with ALS new hope and should stimulate more research in this approach.

8. MSCs EVs in Huntington

HD is an inherited neurodegenerative ailment that results in gradual brain cell death in the putamen, caudate, and cerebral cortex, causing physical, mental, and emotional complications. It is induced by dominant mutations in the IT-15 gene which causes the synthesis of huntingtin protein with poly-glutamine (polyQ) repetitions by increasing the amount

of CAG repeats inside an existing polyQ repetition gene sequence. Huntingtin interacts with about 100 additional proteins, suggesting pleiotropic activities (177-182). Polyglutamine huntingtin protein is indeed transported to other cells by the exosome in HD. As a result, exosomes are crucial in the advancement of HD pathogenesis. Exosomes-based strategies have been tested in the treatment of HD (183, 184). In this regard, Lee et al. found that exosomes from adipose-derived mesenchymal stem cells (ADMSC) regulate pathogenic characteristics in the in vitro HD models by reducing intracellular aggregates of mHtt and raising PGC-1 and phospho-CREB (cAMP response element-binding) expression levels (185). Another work by the same group found that exosome delivery of miR-124 to the striatum of R6/2 HD transgenic mice had minimal effect on behavior, despite a drop in the intracellular expression of REST, which is a miR-124 targeted gene (186). Studies have shown that MSCs-EVs have particular effects on HD.

9. Challenges

MSC-EVs have comparable therapeutic properties to their parental cells, MSCs, but they have certain advantages over MSCs since they lack nuclei and therefore cannot proliferate or differentiate abnormally. Furthermore, small-scale EV isolated through filtration with 0.22 μm membranes are sterile and do not necessitate further sterilization (187). However, the utilization of these EVs in medical settings will necessitate the resolution of many critical issues, including (i) production in large-scale and isolation methods, (ii) quick and precise characterization and quantification methods of EVs, (iii) specific cargo content characteristics, (iv) transfer mechanisms of EVs towards the target location, and (v) optimal dose determination(188).

A two-dimensional culture method is used in traditional cell maintenance and growth approaches. Long-term passaging to manufacture large amounts of EVs may lead cells to lose their clonal and differentiation potential. As a result, there is a critical need for the discovery of strategies for the efficient multiplication of MSCs in order to mass generate EVs for clinical application (189). Several parameters, including early vs late cell passage, the concentration of oxygen, cellular confluence, heparin, cytokines, and serum content of the medium might influence the quality and amount of EVs generated by MSCs (190). Despite large-scale EV production, there is a lack of scalable methods for EV isolation, rendering clinical translation of EV treatment challenging. There are now several ways for isolating EV; however, there is no cutting-edge technology for isolating EV in sufficient amounts for therapeutic usage (191, 192).

MSC-EV's future relies on MSC culturing on a large scale. This critical phase in the production process will pave the way for them to be regarded as a viable therapeutic alternative. EVs serve as important transporters between MSCs and injured tissues. While MSCs may accumulate in unanticipated tissues, the EV they release is directed at injured areas. More study is warranted to discover more about the biodistribution and targeting of MSC-EV to elucidate their effects. However, no unexpected targeted effects have been recorded in clinical or preclinical investigations to yet. Despite issues with large-scale manufacturing, MSC-EVs are rapidly being explored owing to their minimal complications and a variety of advantages (188).

10. Conclusion

MSC-EVs have been shown to have therapeutic promise in a range of tissue injury scenarios. In vivo studies further showed that MSC-EVs are just as effective as their parental cells at stimulating tissue regeneration after treatment. This is intriguing for prospective clinical applications of MSC-EVs, and it demonstrates that MSC-EVs-based treatments may be an affordable choice to MSC-based therapies. However, given the limitations of currently available EV quantification approaches, published studies comparing the effectiveness of MSC-EVs and their cellular counterparts should be interpreted with caution. Similarly, more emphasis should be made on discovering the most effective therapeutic dosages of EVs. Furthermore, there is currently a scarcity of evidence on the long-term impacts of MSC-EVs. It is anticipated that for some forms of tissue damage, several administrations of MSC-EVs would be unnecessary, however for others, multiple administrations may be required. This will raise the patient's burden as well as the therapy's expense. Another issue that has to be addressed before MSC-EVs can be widely used in clinical settings is their heterogeneity. According to existing evidence, the treatment activity of various MSC-EV entities differs greatly. More research is required in this area to establish MSC-EV subgroups with the highest therapeutic potential. Similarly, new methodologies and additional research (in genomics, proteomics, and other fields) are necessary to accurately and specifically define and distinguish EV subtypes. It would also be necessary to improve the reproducibility of relatively large EV production with high purity and specific therapeutic activity. This will entail the development of well-in vitro quality control monitoring systems that are adapted to the unique demands of the condition being treated with EVs. According to current preclinical research employing tagged MSC-EVs, the EVs target macrophages and the wounded tissue of interest. This demonstrates that MSC-EVs' positive impact on tissue regeneration is achieved not just through modulating immune responses around damaged tissues, but also by directly interacting with the tissue. A more in-depth look at this combined MSC-EV activity could help them better target the right tissues and enhance their treatment efficacy. Importantly, elucidating the mechanism of action of MSC-EVs will help determine their legal position and may enhance their therapeutic effects, both of which are crucial for their prospective clinical applications.

Deceleration

Funding

We do not have any financial support for this study.

Conflict of interest

The author declares no conflict of interest regarding the publication of this paper.

Ethical approval

Not applicable

Availability of data and material

The datasets analyzed during the current study are available upon request with no restriction.

Consent for publication

This manuscript has been approved for publication by all authors.

Authors' contributions

All authors contributed to the conception and the main idea of the work. M.M, A.M drafted the main text, figures, and tables. A.M supervised the work and provided the comments and additional scientific information. Z.S.R also reviewed and revised the text. All authors read and approved the final version of the work to be published.

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